Comment on "additive genetic breeding values correlate with the load of partially deleterious mutations"

Postma, E

Abstract: Tomkins et al. (Reports, 14 May 2010, p. 892) reported a strong negative correlation between breeding values and mutational load in cow-pea weevils. Here, I show that this result can be attributed to a statistical artifact. By testing the observed correlation against an incorrect null hypothesis, they find a negative correlation where one does not exist.

DOI: https://doi.org/10.1126/science.1200996

Comment on "Additive Genetic Breeding Values Correlate with the Load of Partially Deleterious Mutations"

Erik Postma

Institute of Evolutionary Biology and Environmental Studies, University of Zürich, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

E-mail: erik.postma@ieu.uzh.ch

Here I show that the strong negative correlation between breeding values and mutational load reported by Tomkins *et al.* (Reports, 14 May 2010, p. 892) can be attributed to a statistical artifact. By testing the observed correlation against an incorrect null hypothesis, they find a negative correlation where none exists.

A correlation between additive genetic breeding values and mutational load is to be expected if genetic variation in traits closely related to fitness is largely the result of partially recessive, deleterious mutations (*1-3*). Estimating this correlation requires knowledge of an individual's breeding value, as well as its mutational load. Predicting breeding values is relatively straightforward when pedigree data is available (*1, 4*), but estimating variation in mutational load among individuals or families, is less so (*5, 6*).

Using an elegant experimental setup, Tomkins *et al.* (*7*) first predict offspring phenotypes (*p*, see *8*) from the breeding values of their outbred but related parents, and compare these to the observed phenotypes (*o*, see *9*) of their inbred offspring. They then use the difference

between the observed and the predicted phenotype ($p - o$) as an estimate of the amount of inbreeding depression shown by a particular family, and thereby of the among-family variance in inbreeding depression (following *6*). Using the amount of inbreeding depression found in the offspring as a measure of mutational load, they then relate $p - o$ to variation in predicted breeding values (i.e. p), and thereby test for a correlation between additive genetic breeding values and the load of partially deleterious mutations.

However, before drawing any conclusions from the strength and direction of this correlation, it is crucial to explicitly formulate the correlation between p and $p - o$ expected under the null hypothesis of no genetic correlation between additive genetic breeding values and mutational load. Tomkins *et al.* state that under the null hypothesis "the observed [inbred family means] are random with respect to the predicted" (as is the case in Fig. 1A). However, this implies that inbred offspring do not resemble their parents, even though the trait is heritable. However, both p and o have an additive genetic component, and hence predicted and observed phenotypes will be correlated (Fig. 1B). This is true, irrespective of whether there is a correlation between p and $p - o$ (*10*).

Unlike p and o, we would at first sight expect $p - o$ and p to be uncorrelated under the null hypothesis. However, according to Tomkins *et al.*, the correlation between $p - o$ and p may well be non-zero in the absence of a correlation between breeding value and inbreeding depression, as "[...] larger values of predicted – observed will always tend to be associated with larger values of the predicted, simply because more minus anything returns a larger number than less minus anything", and this would result in a non-zero correlation between $p - o$ and p. Although correct for two series of random numbers (Fig. 1C), as outline above,

p and o are not independent, even under the null hypothesis. Hence, we cannot treat o as a random number. While at first glance it may appear that by correlating $p - o$ and p we are correlating p with p minus 'something', by subtracting o from p, we are in fact removing the dependence between the two. So, if the null hypothesis is true, the correlation between p and $p - o$ really is zero (Fig. 1D) (*11*).

Nevertheless, when Tomkins *et al.* randomize o relative to p, and subsequently correlate $p - o_{randomized}$ and p, they find a strong positive correlation between the two. This correlation, however, is an artifact, introduced by p and o not being independent. If p is relatively large, we are most likely to draw a random value of o that is smaller than p. Similarly, if p is below average, a random value of o is more likely to be larger than p. Consequently, if p is large, p minus a random value of o is on average large, and if p is small, p minus a random value of o is on average small. Randomization thus generates a positive correlation between $p - o_{randomized}$ and p (*12*). Hence, rather than exposing potential biases, in this case randomization generates a bias that is not there in the original data (Fig. 2).

From their randomization tests, Tomkins *et al.* erroneously conclude that under their null hypothesis, $p - o$ and p are significantly positively correlated. To correct for this, they calculate the genetic correlation between breeding value and inbreeding depression as the correlation between $p - o$ and p, minus the correlation between $p - o_{\text{randomized}}$ and p. However, if the latter is significantly greater than zero, whereas the former is close to zero, this 'corrected' correlation will be significantly negative. Indeed, whereas Tomkins *et al.* found the average correlation between $p - o_{randomized}$ and p to be 0.3, the average 'corrected' correlation between $p - o$ and p was -0.24. Using the correct null hypothesis (i.e. the

correlation between p and $p - o$ is equal to zero) instead, we obtain a mean estimate of -0.24 + 0.3 = 0.06.

Above I have argued that the apparent negative correlation between breeding value and inbreeding depression is an artifact from an overestimation of the correlation between p and $p - o$ under the null hypothesis, and that this overestimation results from p and o not being independent if a trait is heritable. This argument is corroborated by a strong negative correlation between the heritability of a trait and the corrected correlation between p and $p - o$, with traits with the highest heritability having the most negative correlations (r = -0.66, P = 0.006; using estimates from Table S1).

The idea of a negative correlation between additive genetic breeding values and mutational loads is appealing, as it provides an answer to the enigmatic question of how genetic variation is maintained in the face of selection (*13*). Using the correct null hypothesis, the study by Tomkins *et al.* suggests that this correlation is very weak at most. However, more work is required to establish the statistical power provided by their experimental design, and whether the correlation between $p - o$ and p provides an unbiased estimate of the genetic correlation between mutational loads and breeding values for fitness.

References and Notes

1. M. Lynch, B. Walsh, *Genetics and Analysis of Quantitative Traits*. (Sinauer, Sunderland, MA, 1998).
2. M. Lynch, L. Latta, J. Hicks, M. Giorgianni, *Evolution* **52**, 727 (1998).
3. N. H. Barton, P. D. Keightley, *Nat. Rev. Genet.* **3**, 11 (2002).
4. E. Postma, *J. Evol. Biol.* **19**, 309 (2006).
5. M. O. Johnston, D. J. Schoen, *Evolution* **48**, 1735 (1994).
6. C. W. Fox, *Am. J. Bot.* **92**, 1929 (2005).
7. J. L. Tomkins, M. A. Penrose, J. Greeff, N. R. Lebas, *Science* **328**, 892 (2010).
8. $p = \mu + \hat{a}$, where μ is the population mean and $\hat{a}$ the predicted breeding value, given by the mean of the parental predicted breeding values.
9. $o = \mu + a + e - d$, where a is the true breeding value, e the environmental deviation and d the amount of inbreeding depression (i.e. mutational load).
10. If $\hat{a}$ is an unbiased prediction of a, then $\mathrm{cov}(\hat{a}, a) = \mathrm{var}(\hat{a})$ (see *4*) and $\mathrm{cov}(\hat{a}, e) = 0$. From this it follows that $\mathrm{cov}(p, o) = \mathrm{cov}(\hat{a}, a + e - d) = \mathrm{var}(\hat{a}) - \mathrm{cov}(\hat{a}, d)$. So if $\mathrm{cov}(\hat{a}, d) = 0$ (the null hypothesis), then $\mathrm{cov}(p, o) = \mathrm{var}(\hat{a})$.
11. As $\mathrm{cov}(p, o) = \mathrm{var}(\hat{a}) - \mathrm{cov}(\hat{a}, d)$, $\mathrm{cov}(p, p - o) = \mathrm{var}(p) - \mathrm{cov}(p, o) = \mathrm{var}(\hat{a}) - (\mathrm{var}(\hat{a}) - \mathrm{cov}(\hat{a}, d)) = \mathrm{cov}(\hat{a}, d)$. So if $\mathrm{cov}(\hat{a}, d) = 0$ (the null hypothesis), then $\mathrm{cov}(p, p - o) = 0$.
12. As $\mathrm{cov}(p, p - o) = \mathrm{var}(p) - \mathrm{cov}(p, o)$ and $\mathrm{cov}(p, o_{randomized}) = 0$, $\mathrm{cov}(p, p - o_{randomized}) = \mathrm{var}(p)$.
13. I. Booksmythe, H. Kokko, M. D. Jennions, *Curr. Biol.* **20**, R672.

14. I am grateful to L.F. Keller, P. Nietlisbach, B. Tschirren and P.W. Wandeler for comments and discussion, as well as to the Swiss National Science Foundation (SNSF) for funding (grant 31003A-116794).

105

Figure legends

Fig. 1. The relationship between p, o and $p - o$. Unlike x and y (A), p and o are not independent (B). Although x and y are uncorrelated, $x - y$ and x are positively correlated (C), but $p - o$ and p are not (D). p is simulated by drawing 1000 values from a normal distribution with mean of 10 (i.e. μ) and a variance of 5 (i.e. var($\hat{a}$)). o is simulated as $\hat{a} + e - d$, where e and d are drawn from normal distributions with means of 0 and 2 (i.e. mean e and d), and variances of 5 and 2.5 (i.e. var(e) and var(d)), respectively. Note that here the variances of $\hat{a}$ and a are equal, or in other words, that the reliability of the predicted breeding values is 1 (*4*). Furthermore, note that if $\hat{a}$ is an unbiased prediction of a, it follows from (*10*) that the slope of o against p (B) is always equal to 1. To make them comparable to p and o, x and y are drawn from normal distributions with means of 10 and 8, and variances of 5 and 12.5, respectively.

Fig. 2. The effect of randomizing o on the correlation between p, o and $p - o$. Unlike p and $o_{randomized}$, which are uncorrelated (A), p and $p - o_{randomized}$ are positively correlated. The histograms represent the distribution of 1000 values of $r_{p,\ o\ randomized}$ and $r_{p,\ p\ -\ o\ randomized}$, respectively. The dotted vertical lines indicate the value of $r_{p,o}$ and $r_{p,\ p\ -\ o}$ (see Fig. 1B and D). For details on the simulation of p and o, see Figure 1.

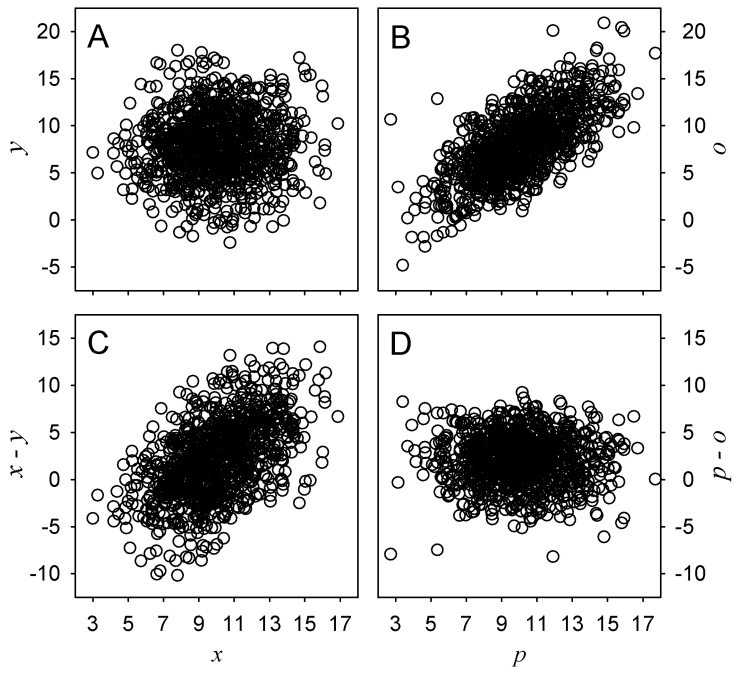
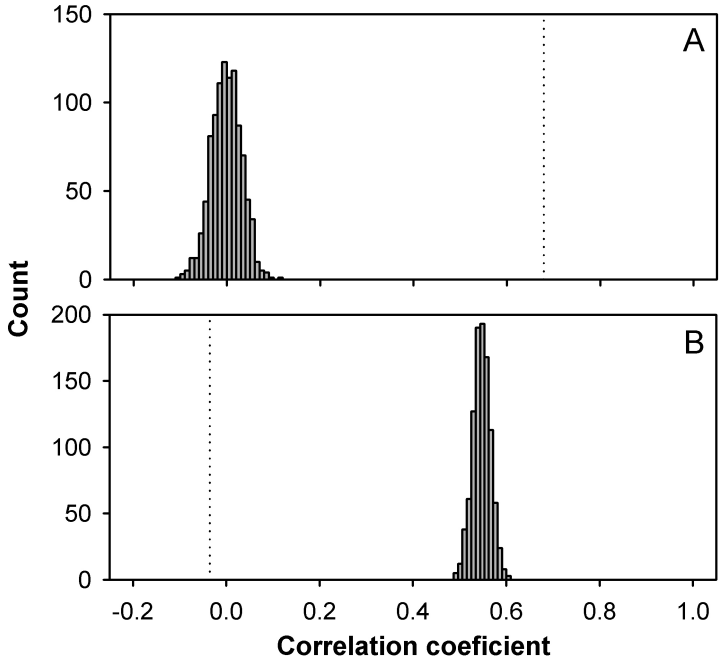


Figure 1


150
100
50
0
A
Count
200
150
100
50
0
B
-0.2
0.0
0.2
0.4
0.6
0.8
1.0
Correlation coeficient

125

Figure 2